SERUM VITAMIN E AND THE SICKLING STATUS IN CHILDREN WITH SICKLE CELL ANAEMIA AS SEEN AT KENYATTA NATIONAL HOSPITAL.

A dissertation submitted in part fulfilment for the degree-of Master of Medicine (Paediatrics and Child Health) Degree of the University o.f Nairobi ~ .1985* ″

by

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This dissertation is my original work and has not been presented for a degree at any other University.

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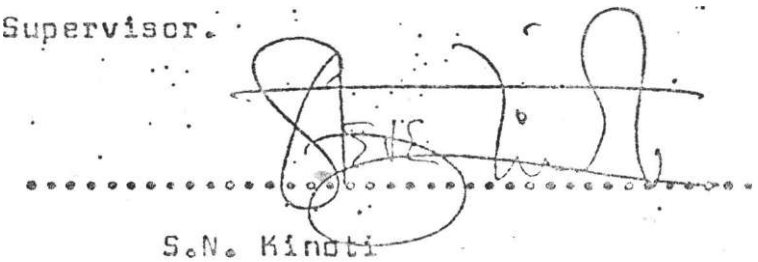

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LIST OF FIGURES

t1ST OF TABLES

LIST OF ABBREVIATIONS

KNH	«	Henyatto National Hospital
PDU	- ,	Paediatric Observation Hard
SCO	-	Sickle Cell disease
SCA	-	Sickle ceil anaemia
DNA	-	Deoxyribonucleic acid
HDL	« ..	High density lipoproteins
• ISC' s		Irreversibly 5'ickled cells
SD	-	Standard Deviation
A	«	Angstrom units

C O N T E N T S :

S U M M A R V s

In this study, scrum vitamin E levels were determined among 62 children with sickle call anaemia end 35 age-matched controls^ Among the sicklers, the irreversibly sickled cell counts were dona and correlated with the serum vitamin E levels©

There was a significant difference in serum vitamin E values ($P < 0*001$)$_t$ the sickle cell anaemia patients showing tendency to deficiency with 27% of them deficient as against $2_0S\%$ of the controls®

It was found that the vitamin E deficient sicklers had a significantly higher irreversibly sickled cell counts (P C 0o02), indicating that vitamin E is an important inhibitor of the irreversibly sickled cell formation®

I N T R O D U C T I O N :

Sickle cell anaemia (SCA) is a genetic disorder caused by a point mutation in DNA (deoxyribo« nucleic acid) that codes for valine rather than glutamic acid, in the sixth position from the N-terminel, of the turn beta-glnbin chains of the heemoglobin tetramer. It occurs with high prevalsnc in tropical Africa and among emigrants from this region. The gene frequency on the continent ranges from and in Kenya from 0-25% among various tribes (1)« It ha3 long bean known as a cause of morbidity and mortality in this region (2). Among patients attending the Kenyatta National Hospital (KNH) Paediatric Haeraatologv Clinic, subjects with 3ickle cell anaemia constitute. two thirds of all cases (3)o

Since the first description of a case of sickle call disease (SCD) by Herrick in 1910 (k) and the upsurge of research and literature, a lot has been uncovered as to the pathophysiology of the disease process in this condition. A large amount of work has gone into a-gtˆmpting to delineate the clinical features and pathophysiology in order to enable development of suitable and rational management of the condition.

The clinical manifestations of sickle cell anaemia are characterised by chronic haemolytic anaemia,

recurrent vasoocclusive painful attacks, frequent bacterial infections and, in some cases, eventual lass of organ function® However, a marked clinical diversity exists in this disease,. For instance, some sickle cell anaeraia patients have many vasaoc« elusive crises and require frequent blood transfusions and hospitalisations while others rarely have campli« cations. Although there is no doubt that the molecular defect of sickle cell anaemia resides in the haemoglobin, additional secondary factors must be sought to explain these diverse clinical manifestations«, One of these factors may relate tu membrane peroxidntive damage and its effects on the pathophysiology of sickle cell anaemia.

Tappel (5) suggested that peroxidative reactions contribute to the degenerative processes that eventually lead to cellular breakdown. Erythrocytes in circulation are especially prone to peroxidative damage because conditions that favour peroxidation are apparently optimal in these cells: possession of a membrane rich in polyunsaturated fatty acids, continued exposure to high oxygen tensions and acting as a vehicle for haemoglobin, one of the most potent catalysts for initiation of peroxidation (6)* The fact that normal cells are protected from peroxidation in vivo is attributable to efficient antioxidant mechanisms:

partly a function of structural, integrity of each cell and partly reflective of the antioxidant systems within the cell. Included are superoxide dismutase, glutathione peroxidase^ catalase and vitamin E„ An impairment of any of these mechanisms may render the erythrocyte more susceptible to peroxidation eventually leading to its breakdown.

ChiUj Lubin and Shohet have demonstrated increased in ijitro susceptibility of the red blood cells to peroxidation in sickle cell anaemia patients (?}<, This increased susceptibility is not entirely due to abnormal membrane lipid asymmetry. It has indeed been shown that even under oxygenated conditions in which: most red blood cells containing sickle haemoglobin are biconcave discs, such erythrocytes are still more susceptible to lipid peroxidation than sre normal erythrocytes. (7)。 This suggests an abnormality in the antioxidant system in addition to that Induced by abnormal membrane lipid asymmetry. Superoxide dismutase, which provides protection for cytoplasmic components against damage by superoxide radicals, was reported by Nair, HcCullough and Das (a) to be"elevated in sickle cell erythrocytes » It has also been shown thst erythrocyte glutathione peroxidase activity elevated in sickle cell anaemia patients and that there is a significant negative correlation between erythrocyte vitamin E and glutathione peroxidase activity in these patients (9)。

The abnormally shaped erythrocytes present in sickle cell aneamia patients regain their normal biconcave shape upon reoxygenation. However, a portion of tha circulating red cell.retain their sickle shape even when fully oxygenated » the irreversibly sickle cells(ISCs)« *he number of irreversibly sickled cells are relatively constant in the same individual although they may vary Prom among patients (10, 11, 12)© The haemolysis observed in sickle cell anemia subjects correlates well with tha percentage of irreversibly sicklad cells (11).

There is evidence that the formation of irreversibly sickled cells is related to changes in the membrane rather than alteration in the haemoglobin structure (13, 14, 15)• During the sickling process, there is net loss of membranes as evidenced by membrane. fra ntation demonstrable by cinematography (15), rearrangement of membrane lipids(7), changes in tnembrane^haemoglohin interactions (13) and increased potassium and water loss (16). .It has .been suggested that a defect in {spectrin«actin lattice' may be the abnormality of the irreversibly sickled cell membrane accounting for the permanent bizarre shapes of these cells (1*0.

All tha vitaim E in the erythrocytes is located in the membrane (17)«, In vitro studies have shown that

the red blood cells of persons deficient In vitamin E ere more prone to oxidation <16, 19), ere more susceptible to peroxiriative haemolysis (19), more readily feˆm Heinz bodies after exposure to hydrogen peroxide (2D) and are more susceptible to potassium loss (21). Vitamin E apparently stabilises the erythrocyte membrane against oxidative stress probably functioning as a free radical scavenger (22) and also structurally stabilising the erythrocyte cell membrane (23).

It has been shown that vitamin E equilibrates rather rapidly uith red blood cells (2'+), henca serum vitamin E levels are a good reflection of the red blood ceil levels.

The foregoing stimulated the author to evaluate the serum vitamin E and the Sickling status among children uith sickle cell anaemia in order to determine its contribution tD the pathophysiology cf this condition..

AIMS AND OBJECTIVES:

1. To determine and compare serum vitamin E levels in children with sickle cell anaemia (haemoglobin SS) and age-matched controls (haemoglobin AA).

2. To determine whether the serum level of vitamin E correlates with the sickling status as determined from the irreversibly sickled cell counts.

MATERIALS AND METHODS:

This study was conducted at the Paediatric Haematology clinic of the Henyatta National Hospital from October 1383 to January 1984. The clinic is held on Monday mornings and handles the bulk of children with haemotologicsl problems that reside in the city of Nairobi, but also handles haematological referrals countrywide. Host children confirmed by haemoglobin electrophoresis tD have sickle cell anaemia that live in or around the City are followed up in this clinic. Most of these children are in steady state and are seen once every 2 to 2 months, the severe forms being seen more ofteˆa. On each visit, the parents are interviewed about the general health of the child at home since the previous visit- and the child reappraised particular attention being paid to fever, jaundice, pallor of mucous membranes, or features of vasoocclusive painfull attacks® Those with Harked pallor of mucous membranes and who on coultergram have a haemoglobin concentration less than 5g/dl, those with severe vasoocclusive painful attacks or with evidence of severe bacterial or other infection are admitted to the hospital's Paediatric Observation Llard (POD!) for appropriate care,. The children in stes Jy state are maintained on daily [1]'ollc acid and proguanil usually obtaining enough stock to last them until the date of the next appointment.

DEFINITIONS:

The following definitions and criteria were used for purposes of this study:«»

1* Sickle cell anaemia (SCA) indicates homozygous inheritance of the sickle cell gene (haemoglobin SS)« All red cell3 in these patients contain sickle haemoglobin®

2. Normal vitamin £ levels were taken as 5^20 micrograms per ml; levels below this range reflect deficiency (25).

3. The irreversibly sickled cell count is the percentage of sickled cells after equilibration of the blcud with oxygen Co full oxygenation.

Clinical Methods-:

In the study period, 5 children on fallow up for sickle cell anaemia who were the earliest to register at the clinic on each clinic day were entered into the study provided they satisfied the fallowing:

1. Had no clinical evidence of crisis: hyper-haamolytic,, vasoocclusive or sever₂ infection<,

2, Had not had a blood transfusion in the preceding 3 months.

3„ Were not on medications known to affect liver function e.g. phenothiazines, barbiturates.

k. Were above the 80% line of the 50th centile of the Havard standards for ᵁᴮ, nht f«

For controls, age-matched children attending the general Paodiatric outpatient clinic for siinor problems were used* This is a busy clinic running every day frsra 8.00 am® to 5&00 pra. The author attended the clinic on yednesday nomlngs, whenever possible, to see children alongside the medical officers working in the unit. Every second child lined up for the author would be entered into the study provided they satisfied criteria 2, 3 and k as for sicklers, and had haemoglobin AA on haemoglobin electrophoresis.

For each of the children recruited into the "study, the author took record of naae, sex, a brief history including frequency of crises, hospitalisations and date of last blood transfusion. He then carried out a clinical examination and., took record of the nude body weight. After obtaining informed consent, 7»5 ml of blood was drawn by venepuncture under aseptic conditions and divided into 2 portions^-

1. 2 mis was put into a sequestrene bottle which was gently mixed and labelled. This was destined for peripheral blood film for reticulocyte and white blood cell differential counts, irreversibly sickled call counts, estimations of haemoglobins F and A„ o

2» 5.5 ral was placed in a universal battle covered with aluminium foil to prevent photo-degradation, This was deep frozen at «20°C until vitomin E assays were done®

Laboratory Methods:

The laboratory tests and essays were done by competent laboratory technologists in haematology and chemical pathology in the Nutrition laboratory of the Medica'l Research Centre, Kenya Medical Research Institute, Nairobi.

1. Haemogram was done by use of Coulter counter Model 'S¹.

2o The haemoglobin was characterised by electro-phoresis on cellulose acetate paper at pH 8.6.

3, Thin blood films were prepared and stained with Jenner-Giemsa stain and reticulocyte and white blood cell differential counts done by light microscopy.

k. Haemoglobin F was measured by alkali denaturation[38*] method (26>«,

5. Haemoglobin ft^ was measured by the cellulose .acetate eiution technique,,

S« The irreversibly sickletf cell count urns done using a modification of the method of Gerties find-Milner (27)® 0*5 ml of anticoagulated

blood UBS spun at 15DD rpm, cells and plasma were reconstituted to haematocrit of 25% and equilibrated uith *95%* oxygen and 5% carbon dioxide for ID minutes. Uery thin blood films isjere immediately prepared and stained by the Jenner-Giemsa stain and the irreversibly sickled cell count determined by counting 500 red blood cells by light microscopy.

Serum vitamin E uas determined using the Aminco-Bouian spectrophotofluorometer after the method of Hansen and Warwick (2B>«

Clinical and laboratory data were recorded on the patient's data sheet appendix I.

Statistical Methods:

Student's 't' test for paired comparison and the conditional test on means were used®

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RESULTS: —>TY1 PF NAlKOBi

A total of 62 sickiers and 35 controls mere enterred into the study* Among the sickiers the irreversibly sickled cell count uas possible in h9„ The complete data on various indices are presented in appendices IIA and XIB.

Figure I show3 the scatter of the serum vitamin E levels among sickiers as compared to the controls. Also shown in the figure ere the 95% confidence intervals for patients and controls₀ The bands are evidently distinct depicting in a picturesque manner the significant difference that exists in the 2 groups of children.

Among the sickle cell anaemia children 17 cut of 62 (27.ˆ%) had values of serum vitamin E in the deficiency zone whereas in the controls only 1 out of 35 (2.₀9%) was in the deficiency zone®

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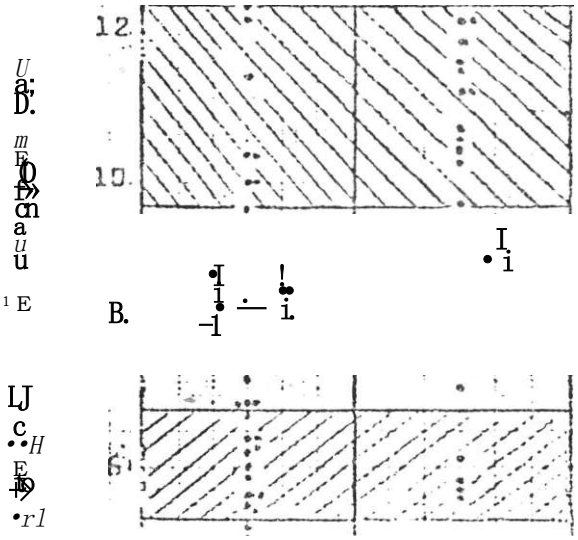

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E S v 9^ ''cQr, fidence interval for control::

^ . . . : . I. confidence intf?rval for SC/″!.x:hil. -<:n

•vp: uc:' beloili dotted line reflect deficiency.

Figure 2 shous the relationship between serum vitamin E and age in a scatter diagram. 95% confidence intervals are likewise indicated for sicklars and controls® There is no significant variation of serum vitamin E with age among both the sicklers and controls.

Figure 2 sheus the relationship between serum vitamin £ end ege in a scatter diagram. 95% confidence intervals are likewise indicated for sicklers and controls*. There is no significant variation of serum vitamin E with age among both the sicklers and controls.

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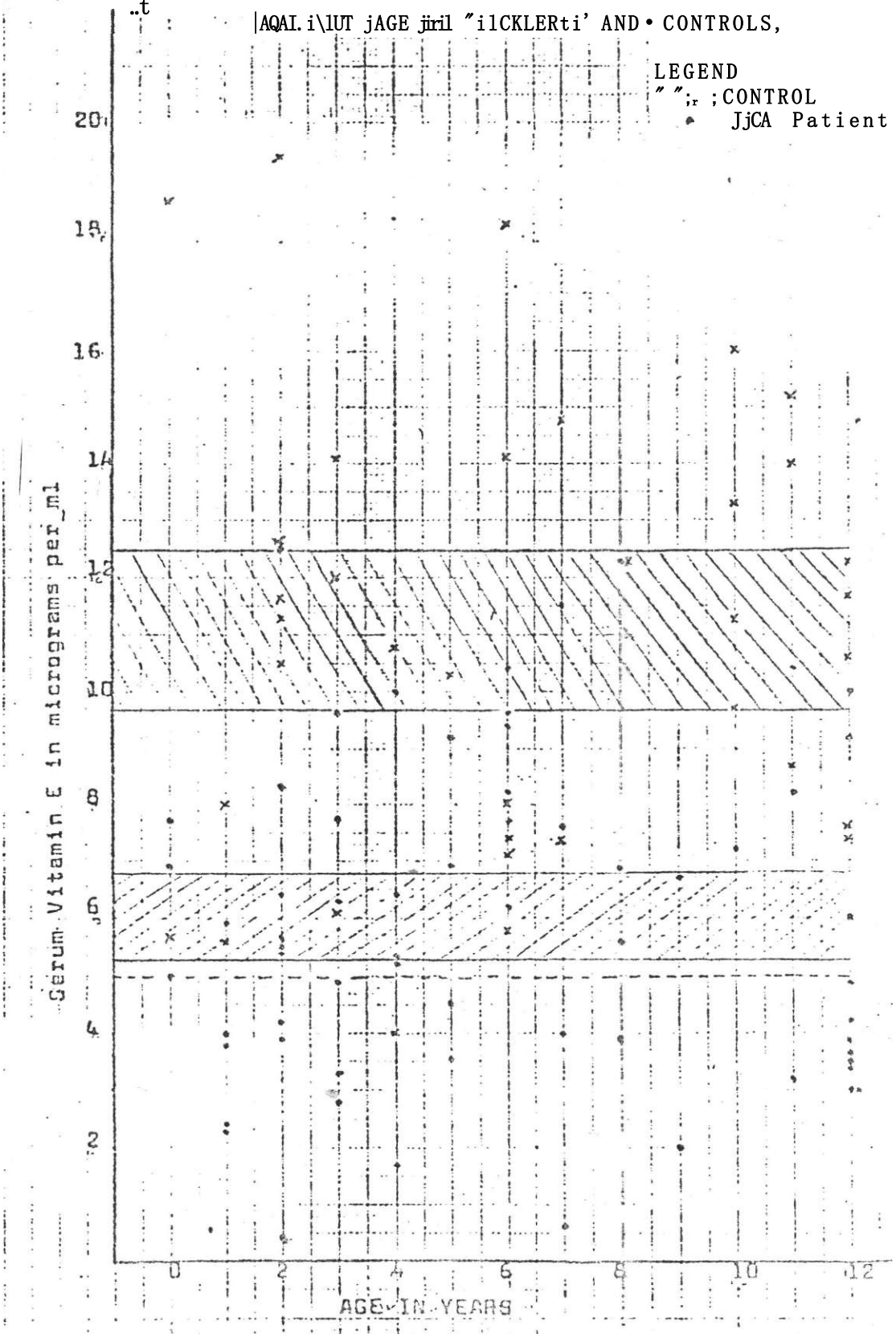

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Table I: \JITAMIN E LEVELS IN THE SICKLE CELL ANAEMIA PATIENTS AND CONTROLS

SUBJECTS	o	Sarum vitamin E in Eiicrograms/ml	
		Range	Mean + ISO
SCA patients	62	0«4 « 12.5	6.06 • 2.91 tt*
Normal cont-rols	35	4.0 - 19c4	Hell +

In the population of £2 patients, ranging from age of 6 months to 12 years the serum vitamin E levels varied from 0。4 •• 12.5 micrograms per ml with a mean of 6.06 £ 2<>91. The ranga for the 35 age-matched controls u;as 4.0 - 19.4 micrograms per ml with mean of 11d1 + 4c14„ The difference in means is statistically significant (P<0。001)。

Table 2: FREQUENCY DISTRIBUTION OF VITAMIN E LEVELS IN THE SICKLE CELL ANAEMIA PATIENTS AND CONTROLS

	CLASS	PATIENTS	CONTROLS
VALUE	Mg/ml		
1	0-19©	2	
2	2~3„9	16	
5	^-5.9	15	k
7	6-7 o9	Ik	6
9	6-9₀9	a	⊄
11	1S-11o9	5	7
13	12-13c9	2	5
15	14-15.9		5
17	16-17꜀9		1
19	18»19₀9		3
21	20-21.9		
	n	62	35
	5"	376	389
	X	6.06	Hell
	a	₈SX	

jfabie 2 shows: the frequency distributions and the statistical analysis. P< 0«001 and the 99% confidence interval of difference 2.90 - 7*20

Table 3: THE IRREVERSIBLY SICKLED CELL COUNTS IN VITAM E DEFICIENT SICKLERS AS COMPARED TO SICKLERS WITH NORMAL LEVELS

Vitamin E j	<5Mg/ml	^ 5h g/ml
n	17	32
Range ISC	2,2- 28	0o2-19o2
Mean	9.37	
Mean of transformed data	2.85	2.02

t value of difference 2<>65

P <0„02

Table 3 shows a comparison of irreversibly sickled cell count among children with sickle cell anaemia who had norma!values of serum vitamin?end those who showed deficiency of this vitamin. There Is shown to be a stitisticaliy significant elevation of the irreversibly sickled cell count in vitamin £ deficiency <P C 0*02)

The whole blood haemoglobin concentration ranged from 5*1 « 10₀7 g/dl with amean of B.Qg/dl. There wao a slight positive, but not statistically significant correlation £0.60)ₜ between serura vitamin E and whole blood haemoglobin concentration. This means that the vitamin E deficient patients tended to be more anaemic© There also was a slight negative, but not statistically significant correlation (r=-0,3B)ₚ between serum vitamin E and the reticulocyte count• This means that the vitamin E deficient children tended to have higher reticulocyte counts, an evidence of Increased haemolysis in these children,.

The range of sickle haemoglobin among 55 children for whom it was determined, was 77.2-96.2% with a mean of fig/l/b. The range of haemoglobin F was 1„*+«20,»5% with a mean of 7*0%₀ Haemoglobin Aˆ ranged from 0.S˜3o9% with mean of 1ₛ7%„

DISCUSSION:

The results of this study show that children with sickle cell anaemia have significantly low serum vitamin E levels* The 62 children with sickle cell anaemia had serum vitamin E levels that ranged from 0.^~12.5 micrograms per ml, with a mean of 6„QS 4- 2.91„ 17 (27„t+%) of these children had levels of vitamin E that fell in the deficiency zone. The 35 age-matched controls.; on the other hand, had serum levels of vitamin E that ranged from U»0 ~ 19*5 micrograms per ml with a mean of 11d1 + ^<>1^. The difference in the means is statistically significant (P< 0*001). Tteee results are in agreement with those of Chiu and Lubin ;(g) although these investigators had oni.'g- 16 patienta in their study and analysed erythrocyte levels of vitamin E rather than serum levels„

The aeticlogical factors leading to low serum vitamin E levels®in patients with sickle cell anaemia are not clear. However a number of possibilities exist. They include reduced, intake, poor absorption and increased utilisation.

Since controls in this study were selected from the same catchment area for Henyatta National Hospital, there is no reason to believe that the differences in vitamin E levels reflect differences in dietary intake as it is presumed that these groups ere within the same social class and their eating habits are similaro

Rosenblate (29), Bogoch (30) and Song (31) in their various papers showed that subjects with sickle cell anaemia suffer marked hepatic dysfunction as shown by altered liver function tests pointing to parenchymal damage, biliary and canalicular stasis and impaired bile salt excretion® In the absence of bile salts, th? absorption of vitamin E is markedly. Impaired (32).

In a recent study, Hinoti, Ndombi and Kyobe (33) have observed decreased serum levels of high density lipoproteins (HDL) In the serum of children with sickle cell anaemia as compared to normal controls,, This observation is similar to observat Ions made for cystic fibres is patients (3^). Since HDL is known to be important in vitamin E absorption (35), and it has been suggested that HDL-vitamin E is probably the

metabolicslly important form of tha vitamin^ with HDL being able to transfer and accept vitamin E from red blood cells to a greater extant and at a faster rate than other lipo-proteins (35)5 low vitamin E levels in serum of sickle cell anaemia patients may be secondary to HDL deficiency in these subjects. HDL deficiency may result fro^ liver dysfunction©

Another contributing factor to the low vitamin E levels may be increased utilisation in serum. Haemoglobin, as found in plasma in sickle cell anaemia patients as a result of intravascular haemolysis, Is known to be a powerful catalyst of lipid peroxidation (6). The plasma haemoglobin might therefore catalyse the oxidative destruction of vitamin E and account for lower serum vitamin E le%>®ls iw.-thnsa patients,,

The results of this study also shewed that vitamin E deficient sicklers had higher irreversibly sickled cell counts than the sicklers with normal levels. Among the 49 patients in whom the irreversibly sickled cell count was done, the 17 (34%) who had deficiencyof vitamin E had irreversibly sickled cell count ranging from *2t, 2»28%* with a mean of 3c31%m ^he remaining 32, whose values for vitamin E

fell In the normal range had irreversibly sickled cell count ranging from 0.2-19.2%with mean of The difference in means of these -2 groups of aicklers was statistically "significant CP < 0.02).

Structural abnormalities such as increased numbers of distorted and contracted cells have been found in vitamin E deficient premature infants (36). Shortened red cell survival has been reported in vitamin E deficient udults (37), vitamin E deficient premature infants (3S) and cystic fibrosis patients with low blood vitamin .E levels (37). ˆhe haeraatological abnormalities observed in these vitamin E deficient states can be corrected by vitamin E supplementations (36,* 37, 38). Taken together, these findings suggest that enhanced susceptibility of sickle cell erythro-cytes to peroxidation may be a factor that contributes to shortened lifespan of the red cell characteristic of sickle cell anaemia.

In addition to accelerated red cell destruction, peroxidative damage may play a significant iole in the pathogenesis of irreversibly sickled cells。 As earlier statedˆ the irreversibly sickled cell in morphologically identified by its failure to regain its biconcave disc shape when fully

oxygenated end is mechanically identified by its entrance fragility and rigidity®

Although there is no correlation between percent irreversibly sickled cells in peri« i pharal blood and vasoocclusive episodes Serjeent cr, ai (11) shewed direct relationship between percentage of circulating irreversibly sickled calls and extent of haemolysis.

The mechanism of irreversibly sickled cell formation is presently unknown. Current concepts of irreversibly sickled cell formation focus on irreversibly deformation of spectrin-actin lattice- the membrane skeletal proteins of the red cell - and on cellular dehydration secondary to abnormal membrane permeability. Studies by Lux, John and Harnorsky (1*0 indicate that this deformation is not dependent upon the persistant interaction bdtween sickle haemoglobin and the spectrin-actin lattice. These investigators suggest that the irreversibly sickled cell formation is the result of *b* perraenent alteration in the spetrin-actin lattice. On the other hanri$_f$ studied by Clsrk$_f$ Mohandas and Shohet C^G) indicate that reduced defamiability of the irreversibly sickler cell is mainly due to dehydration of these cells and that the abnormal deformability can he returned

to normal fallowing cellular rehydration,,
The findings of these investigators imply that the inability of the irreversibly sickled cells to return to their original biconcave ahˆpe is due to a high internal viscosity which is resultant from dehydration caused by abnormal membrane permeability.

These two theories do not have to be mutually
j
exclusive. It is possible that peroxidation damage can lead to permanent alteration of the spectrin-actin lattice as well as i,n abnormal membrane permeability. Possibly, the structurally normal spectrin-actin lattice network is passively deformed by oriented haemoglobin S microfilaments later becoming fixedly deformed. In the case of abnormal membrane permeability, it is possible that peroxidative damage effects membrane components required to maintain normal permeability. Such damage could alter cation transport, create 7QA holes in the membranes as suggested by Jacob and Lux (£+1) and lead tc abnormal passive ion tranport. A comparison of several properties of vitamin E deficient red calls following peroxidant injury with those of irreversibly 3ickled ceils may p: ovide some support into this view。

If vitamin E plays a role in inhibition of formation of the irreversibly sickled cells, then vitamin E supplemental.ion to sickle cell anaemia patients should reduce the irreversibly sickled call counts in the peripheral blood• This is exactly what- Natte, Mechlin and Brin (2,3) end Kinoti, Heme, Buibc and Daws (42) found in their etodies* A double blind crossover study is currently under way at the Paedistric Heematology' clinic to evaluate the role of vitamin E supplements on the irreversibly sickled call count in sickle cell anaemia patients.

The results of this study confirm that peroxidsnt damage secondary on vitamin E deficiency, is a significant factor in the pathogenesis of the irreversibly sickled cell formation end • tience '"lft- the pathophysiology of sickle ceil anaemia..

CONCLUSIONS;

From the results of this study the following inferences are noted:

1» It was established that there uas very significantly low levels of vitamin E in children with sickle cell anaemia as compared with normal age-matched controls. Possibilities of this increased deficiency point to malabsorption and overutilisation.

2. Vitamin E deficiency wa3 correlated with significant elevation of the irreversibly sickled cells. It can be inferred that vitamin E is an important inhibitor of irreversibly sickled cell formation.

RECOMMENDATIONS;

lo Sickle ceil anaemia patients and their parents should be givan instructive Nutritional Education ta iraprove vitamin E intake and possibly be supplemented to provide minimal daily requirements.

2. A study to evaluate the interaction of vitamin E and other factors knoun to affect the pathophysiology of sickle cell anaemiij as zinc and fetal haemoglobin levels.

ACKNOWLEDGEMENTS:

1« To my supervisor Dr. S.N. Hinoti whose advice was always invaluable through the whole study's much thanks.

2₀ To all the sisters, Paediatric Haerriatcloty clinic and the Registrars and consultants who worked in this clinic during the time of the study for their cooperation*

3. To all the mothers and the children whose co-operation made this study possible, my sincere sppreciaticn.

The Kenya medical Research Institute (K.E.M.R.I.) for availing their laboratory facility to me for the study, thanks,

5. To the very .competent laboratory technologists who took up their time and made the many tests and assays used in this study a success, particularly Nina Desai, Maria Mwita, Een Omondi, and Oohn Kyobe,,many thanks.

6. To Mr. Gemert, statistician at the Medical Research Centre, K.E.M.R.I., Nairobi, who did the statistical analysis.

7® To Mrs, J. Thairu who patiently did the typing of this work, sincere gratitude.

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APPENDIX I - PATIENT'5 DATA SHEET:

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HAEMOGLOBIN F

HAEMOGLOBIN

HAEMOGLOBIN S

VITAMIN E

RESULTS OF LACORATORY INDICES FOR SICKLERS

No.	INITIALS	AGE Years	SEX	GENOTYPE	HAEMOGLOBIN TYPE % OF TOTAL HbF	HbA	HbS	HAEMATOCRIT	HAEMOGLOBIN g/dl	ISC's %		
1	JAO	9.0	F	SS	7.9	1.3	90.8	21	7.0	2.4		
2	AK	7.0	M	SS	7.8	3.2	89.0	20.3	6.7	-		
3	FOO	12.0	M	SS	4.1	2.4	93.5	21.4	7.2	-		
4	SAO	5.0	F	SS	2.8	3.5	93.7	26.1	8.6	-		
5	OAG	1.8	F	SS	9.3	2.0	88.7	22.9	7.2	-		
6	OJA	0.8	F	SS	9.7	0.8	89.5	32.8	10.7	1.6		
7	RJO	5.0	F	SS	16.0	2.0	82.0	22.1	7.0	2.8		
8	CO	12.0	M	SS	3.6	2.4	94	24.2	8.3	14.4		
9	SO	12.0	M	SS	3.2	2.8	94	28.0	9.4	-		
10	SOZ	2.0	M	SS	5.3	3.6	91.2	24.5	7.6	3.6		
11	AKO	9.0	F	SS	3.1	1.7	95.2	30.4	10.2	-		
12	LJO	2.0	M	SS	6.7	3.2	90.1	26.2	7.6	-		
13	OSB	3.0	F	SS	6.7	3	90.3	26.0	8.7	7.2		
14	PO	12.0	F	SS	2.3	3.4	94.3	19.2	6.8	28.0	3.0	22
15	ASA	3.0	F	SS	12.4	2.7	84.9	28.4	9.7	-	3.3	4.6
16	NBO	1.7	M	SS	1.6	-	-	17.8	5.2	4.2	2.4	14
17	GOO	3.0	M	SS	3.7	3	93.3	22.8	7.4	4.6	4.9	26
18	OO	12.0	M	SS	6.9	-	-	23.8	8.3	8	3.4	19
19	FA	7.75	F	SS	4.1	-	-	28.5	9.5	-	8.3	5
20	ZO	12.0	M	SS	4.6	-	-	22.4	7.7	-	4.2	16.5
21	SO	4.0	M	SS	1.4	-	-	23.8	8.5	26.8	1.7	28
22	GMM	9.3	M	SS	2.4	3.4	94.2	20.2	7.1	13.6	6.7	30
23	LNJ	4.0	F	SS	11.8	2.0	96.2	20.9	6.6	2.6	6.4	31.5
24	OO	11.0	M	SS	8.7	2.5	88.8	20.6	6.8	6.0	3.2	2.4

APPENDIX II A (continued)

NO.	INI11ALa	AGE (year	SEX	GENOTYPE	HAEMOGLOE IN TYF-E % of TOT/1L HbF	HbA?	HbS	HEMATO-CRIT %	HAEMOGLOBIN g/cJl	I%C S	VITAMIf 1: Micrcgrani2; ml	q»7TCs %
25	CD	•8,5	M	SS	6.5	3.2	90,3	22.3	7.7	6.2	5.G	17
HC	OBD	:i2„o	M	S3	4.6	2.4	93.0	2C.6	6.8	6,0	3.6	ic
27	DAA	4.5	F	ss	17	209	80.1	23,0	7.1	2.0	"— *	19
23	OLA	2.0	F	SS	8.	.28	8902	17.7	5ol	3,5	004	33 ’
29	OLA	2.0	F	S*6*	8.6	0,8	900G	25.5	8.4	6,0	5.6	14.5
3ft	0 AO	4,0	M	SLi	5.7	2„0	92.3	23.3	7.8	9.0	4,5	1 c
31	FOO	i.75	M	SS	a.5	1.8	89.7	2004	6.5	9,2	2.3	26
;32	NAO	0 >7	F	SS	21.’	2.4	75.9	24.4	8.1	*0.6*	7.7	3.5•
.’33	KOR	0,5	M	ss	20.1	2.3	77.2	24.6	8.4	1.2 i	6.9	5
;34	0 AO	1.6	M	SS	9.7	3.0	87.3	18.5	6.8	9.0 1	3.8	17 .5
35	ROO	G.5	M	SS	a„g	3.9	87.2	22.9	7.1	4.0	6.2	32.5
″36	OVA	2,7	M	ss	16.t	3.4	80.2	24.6	7.8	4.2	4.2	16.3
37	OLD	12	H	’ ss	4.7	3.2	92,1	26.2	8.3		9.2	15
’33	GOG	0.0	M	ss	3.4	2.7	53.9	24 .2	7.7	9.2	6.9	10
39	OE	5.5	M	ss	6.1	3.2	90.6	27.2	9.6	-	9.2	33.5
40	ABH	7 ,0	r	ss	3.6	2.1	94.3	27.7	9.2	19.2	7.6	2<- .5
41	LA	0,0	F	ss	4.0	4.0	92.0	27.8	8.6	4„S	9.6	12.5
’42	VOM	3.5	M	ss	9.7	2.2	88.1	26.1	900	2.6	$. 2	19,6
43	JAO	2.5	F	ss	12.°	3.2	83.9	29.3	9.6	2.8	6.4	21
y	RMG	6.0	F	ss	5.4	3.8	90o8	23.9	8.4	9,6	9.6	25
45	F A3	12.D	p	ss	3.5	2.4	34.1	22 ″	7„1	1.4	10.0	20

APPENDIX II A (continued)

No.	INITIALS	AGE (year	SEX	GENOTYPE	HA EMOGLOBIN TYPE % CF HbF	TQTAL HbAf, HbS	HAEMATO-crit c	HAEMOGLOBIN g/dl	JJC« S %	VITAMIN E Micragram 3 ml	jPETICS
kS	000	6.0	F	jS	3.0	2.6 93.k	• 20.U	6.3		″3.0	
k7	CO	12o0	F	JJ	3.6	3.a 92	2ˆ.5	8.6	20.6	*U.9*	
UQ	BA	2.0	F	03		2.5 -	19.1	5.6	5.6	*5.k*	25
U9	DO	a.o	M	BS	3,5	3.6 92.9	26.7	8.6	5.2	12.3	12
50	CK	6.0	F	33	9.6	2.7 37.7	29.2	9.5	3.0	7.7	13.3
51	NM	11.3	p	as	*k.t**	3.5 92.1	27.8	9.0 ,	«».0	10.	1'<.6 ,
52	SO	5.0	H	SB	*k.Z*	-	23.2	7.6	13,0	6.9	31.6
53	ADO		M	S3	13.5	1.5 G5	28.6	8.8	! 7.0	10.0	25.0
5k	LOO	3.0	F	S3	10.5	3.2 76.3	2ˆ.5	7.3	0.2	7.7	*2U*
55	LO	12.0	F	SS	5.7	2.9 91.*k*	28.1	9.6	6.2	3.9	27
56	DO	11.0	M	S3	l».B	3.5 91.7	26.2	8.6	12.8	8„2	10
57	BAD	3.7	F	33	11.7	3.2 65.1	2ˆ.8	7.8	2.0	6.36	
58	FO	6.3	M	33		2.3 92.8	25.0	8.5	12	8.2	16
59	DMA	1.6	F	SS	is.;	2.a 81.9	26.ˆ	8.6	G.U	5.9	17
fin	BAU	8.0	*f*	33	10,'	2.2 87.*U*	23.0	7.7	_	3„9	16
61	RAO	6.C″	F	J	13.C	2.6 *8U.U*	2ˆ.8	6.3	2.◇»	9.'4	19''
62	CLA	2.5	F	33	9.7	1.7 89.6	2ˆ.1	7.3	2.◇»	12.5	20

APPENDIX II B: RESULTJ OF INDICES FOR CONTROLS

NO.	INITIALS	AGE	SEX	GENOTYPE	HAEMATOCRIT	HB(g/dl)	V/ITAMIN E Micrograms/ml
1	JA	0.9	F	AA	29.5	6.1	5.71
2	JS	3.0	F	AA	[illegible]	12.5	12.0
3	FO	7.0	M	AA		14.4	7.4
4	RU	10.0	F	AA	49.2	16.0	9.7
5	LM	12.0	F	AA	43	14.2	7.4
6	KM	4.0	F	AA	38.3	12.9	10.8
7	CO	• 4.0	M	AA	34.9	9.5	4„0
6	MN	10.0	M	AA	41.3	13.3	16.0
9	• PM	6.0	M	AA	39.4	12.8	7.42
10	NC	6„0	F	AA	29.5	8.0	8.0
11	AW	8.0	F	AA	42.5	13.5	12.3
I*	HO	12.0	F	AA	32.9	10.2	11.7
13	VW	2.5	F	AA	38.0	11.5	10.5
lit	3M	11.0	F	AA	39.3	11.7	8,7
15	SC	12.0	F	AA	33.2	10.2	10.6
lfi	MM	2.0	F	AA	28.4 .	a.6	12.6
1.7	SS	5.0	M	AA	35.6	10 o7	10„3
ia	MK	1.3	M	AA	29.2	8.2	5.6
19	AW	12.0	F	AA	41.9	14.0	7.6

APPENDIX II B (continued)

NO.	INITIALS	AGE	SEX	GENOTYPE	HAEMATDCRIT	HbCg/dl)	VITAMIN E Micfcograms/m[1]
20	FH	ID. a	M	AA	40.8	13.4	13.3
2 I	BM	3..Q	M	AA	37.5	. L *id#X-*	t5« X
2 2	MIA	6,0	F	AA	3404'	9.9	IB„2
23	1H	a₀a	H	AA	30.8	9*1	11.5
Z.FC	F 0	2.5	H	AA	21.7	7,0	19,4
2 5	TA	2.0	F	AA	40.6	13.1	16»5
	bl'iJ	1.D	F	AA″	32.7	9.7 •	8,0
2 ?	WM	7.0	F	AA	• 39C3	13.1	14.8
	MM	11.0	M	AA	' 38₀4	12.0	15.2
2-0	CK	10.0	M	AA	39.8	12.5	11.3
J30	SO	3.0	M	AA	41.5	12.3	14„1
31	NR«IK	GOO	M	AA	30.8	1105	14.1
	GN	6.0	H	AA	35.8	14₀4	5.8
3 3	HJ	6.0	F	AA	31.6	11.7	7.1
3 4	KFT	1200	H	AA	4 2.2	15.4	12..3
35	FG	11.0	M	AA	35.6	12.4	14.0